AD-A121 891 ACUTE ORAL LETHAL DOSE (LD50) IN MALE AND FEMALE RATS FOR CHR 8(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA M A HANES ET AL. SEP 82 LAIR-129 1/1

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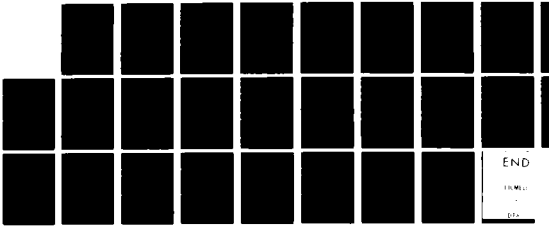

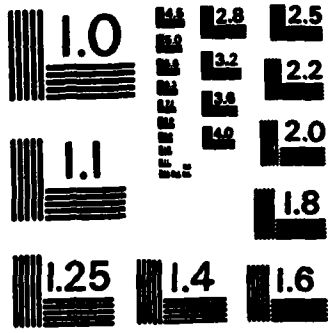

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NATIONAL BUREAU OF STANDARDS-1963-A

INSTITUTE REPORT NO. 129

ACUTE ORAL LETHAL DOSE (LD_{50}) IN MALE AND FEMALE RATS FOR CHR 8

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and
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TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

SEPTEMBER 1982

Toxicology Series- 25

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Oral Lethal Dose (LD_{50}) in Male and Female Rats for CHR 8 (Toxicology Series 25)--Hanes et al

UNCLASSIFIED

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CHR 8, LD_{50}, Acute Oral, Insect Repellent

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The acute oral toxicity potential of CHR 8, a candidate insect repellent was tested in 40 young adult Sprague-Dawley Rats. Doses ranging from 1620 ul/kg to 5250 ul/kg body weight were tested based on an approximate lethal study. Probit analysis was used to derive values for the median lethal dose (LD_{50}). Animals were single dosed and observed for 14 days. The LD_{50} for males was 4809.9 ul/kg with a 95% confidence range of 4035 - 5733 ul/kg. The LD_{50} for female rats for CHR 8 was 3136.5 ul/kg with a 95% confidence interval of 2654 - 3706 ul/kg. Corn oil was the vehicle control and diluent. The toxicity of this chemical is

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in the moderate to slightly toxic range. Continuation of planned human use experimentation is warranted.

ABSTRACT

The acute oral toxicity potential of CHR 8, a candidate insect repellent was tested in 40 young adult Sprague-Dawley Rats. Doses ranging from 1620 µl/kg to 5250 µl/kg body weight were tested based on a approximate lethal study. Probit analysis was used to derive values for the median lethal dose (LD_{50}). Animals were single dosed and observed for 14 days. The LD_{50} for males was 4809.9 µl/kg, with a 95% confidence range of 4035 to 5733 µl/kg. The LD_{50} for female rats for CHR 8 was 3136.5 µl/kg with a 95% confidence interval of 2654 to 3706 µl/kg. Corn oil was the vehicle control and diluent. The toxicity of this chemical is in the moderate to slightly toxic range. Human use experimentation is warranted as planned.

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Report

TESTING FACILITY: Toxicology Group, Division of Research Support,
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Division of Cutaneous Hazards
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

PROJECT/WORK UNIT/APC: Prevention of Military Disease Hazards
3M16770A871, WU 201, Development of Repellents
Against Medically Important Arthropods, APC FL07

GLP STUDY NUMBER: 81022

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Martha A. Hanes, DVM, VC

PATHOLOGIST: MAJ Glen E. Marrs, DVM, MS, VC, Diplomate of American
College of Pathologists

STATISTICIAN: Virginia L. Gildengorin, PhD

REPORT AND DATA MANAGER: Carolyn M. Lewis, MS

REPORT AND DATA MANAGEMENT: All raw data, a copy of the final report,
study protocol, and retired SOPs will be
retained in the LAIR Archives.

TEST SUBSTANCES: CHR 8, a proprietary compound in efficacy testing
for insect repellent properties by the Division
of Cutaneous Hazards.

INCLUSIVE STUDY DATES: 24 August 1981 (limit test)
13 August - 9 September 1981

OBJECTIVE: To determine the acute oral toxicity potential of CHR 8.

ACKNOWLEDGMENTS

The authors wish to thank SSG Lance White; SP5 Joe Alletto, BS; SP5 Marlin McKinley, BS; SP5 Florence McKinley, BS; SP4 Thomas Kellner, BS; SP4 Lawrence Mullen, BS; SP4 Evelyn Zimmerman, William Langley, MS, and Callie Crosby, BS, for performing the daily observations, maintaining the health care of the animals, compiling data in a usable form, and their never ending pursuit of TOXSYS operational quality.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 81022 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Medical Laboratory Studies, outlined by the Food and Drug Administration.

John T. Fruin 26 May 82
JOHN T. FRUIN / DATE
COL, VC
Study Director

Martha A. Hanes 26 May 82
MARTHA A. HANES / DATE
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Glen E. Marrs Jr. 26 MAY 82
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VIRGINIA L. GILDENGORIN, PhD. / DATE
DAC, Statistician

Carolyn M. Lewis 26 May 8
CAROLYN M. LEWIS, MS / DATE
Data Manager

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

17 Jun 82

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81022 the following inspections were made:

20 Aug 81
25 Aug 81, 0845 hours
25 Aug 81, 1500 hours
26 Aug 81
27 Aug 81, 0800 hours
27 Aug 81, 0840 hours

The report and raw data for this study were audited on 16 Jun 82.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Oct 81 report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

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ACUTE ORAL LETHAL DOSE (LD_{50}) IN MALE AND FEMALE RATS FOR CHR 8--Hanes et al

The goal of the insect repellent program is to develop better insect repellents for the protection of soldiers from insects and insect-borne diseases in the field. In the last several years the Division of Cutaneous Hazards, Letterman Army Institute of Research (LAIR), has tested a large number of chemical compounds, submitted by SRI International, the U.S. Department of Agriculture (USDA), and private industry, against a variety of mosquitoes, sand flies, fleas, bugs, ticks, and mites in animals and in vitro test systems. Several of these materials have shown sufficient repellent activity and persistence on the skin of animals to warrant consideration for use in lieu of, or in conjunction with, the current troop-issue insect repellent, 71.25% N,N-diethyl-m-toluamide (m-DEET) in ethanol. The Division of Cutaneous Hazards has also evaluated a number of new formulations of m-DEET prepared at LAIR or submitted by private industry. Several of these new formulations have been more persistent than the current troop-issue repellent in tests on animals.

Toxicity Testing Repellent Program

It is now planned to test the best of the new compounds and formulations on human volunteers to confirm the results that have been obtained in the in vitro and animal tests and to evaluate their performance under conditions of actual use. Before this can be done, it is necessary to obtain certain toxicity data on each compound or formulation to insure that it is safe for application to the skin. The toxicity tests required for registration of a new insect repellent are prescribed by the Environmental Protection Agency (EPA). The basic animal toxicity tests required for experimental use of the new compounds and formulations on human volunteers are prescribed by the LAIR and USAMRDC Human Use Committees. An acute oral toxicity (LD_{50}) test is one of the animal toxicity tests for CHR 8 requested by the Division of Cutaneous Hazards so that the chemical could be considered for human testing. If adverse toxicity data are obtained with the animal tests, the chemical will be eliminated from consideration, and the prospective tests on human volunteers will not be carried out. The toxicity testing program thereby serves as both a safety factor and secondary screen in the repellent development scheme.

Objective of Study

The objective of this study was to determine the acute oral toxicity potential of proprietary compound CHR 8.

METHODS

Test Substance

CHR 8 is a proprietary compound produced by Rohm and Haas; the chemical information has been confidential. For GLP purposes the information is reported as "unknown" because it is unavailable (Appendix A).

Animal Data

Species: Rat (*Rattus rattus*)

Strain: Sprague Dawley

Source: Charles River Breeding Laboratories
3251 Ballard Vale Street, Wilmington, MA 01887

Sex: Male and Female

Age: 6 weeks at receipt

Method of Randomization: TOXSYS^{R} Animal Allocation Program

Animals in Each Group: 7 females and 7 males in each dose group, except for control group containing 5 of each sex.

Condition of Animals at Start of Study: Normal

Body Weight Range: (at receipt) 120-205 g
(at dosing) Males, 180-275 g; Females 161-197 g

Identification Procedures: Ear tag (SOP-OP-ARG-1)

Pretest Conditioning:

a. Quarantine from 13 - 21 August 1981

b. Animals pre-dosed acclimated with 1 cc of water daily from 17 - 21 August 1981.

Justification: The Sprague Dawley rat is a proven sensitive mammalian model for oral LD_{50} determination.

Environmental Conditions

Caging: Number/cage = 1; Type cage used = stainless steel, wire mesh bottom, battery type, no bedding.

Diet: Certified Ralston Purina Rodent Diet 5002 ad libitum.

Water: Central line to cage battery

Temperature: 24 ± 1 C

Humidity range: 44-55%

Photoperiod: 0530 - 2000 hr/day (light, 14 1/2 hr).

Dosing

CHR 8 was removed from refrigerated storage bottles and placed in 20 cc scintillation vials and heated (38 to 42 C) to avoid rapid cooling of the animal's stomach. The volume of pure compound for each animal was calculated and the total volume was adjusted with corn oil to deliver 1.75 ml of total volume per male rat and 1.5 ml per female rat.

Corn oil was used as a vehicle because it allowed the compound to be diluted within acceptable limits, and it has been used historically in LD_{50} studies as a non-toxic carrier for water insoluble compounds.

Results from the Approximate Lethal Dose (ALD) or limit test (study 74001), established the ALD to be between 3190 and 5000 µl/kg for males, and between 2000 and 3130 µl/kg for females. The data for 74001 will be archived with the raw data for this report. The LD_{50} and slope determination was derived by Bliss probit analysis, as described by Finney (1). Methods and results are reported later in this report.

Five dose levels (2290 µl/kg, 2820 µl/kg, 3500 µl/kg, 4270 and 5250 µl/kg) were given to male rats (Table 1). Females received different dose levels (1620 µl/kg, 2000 µl/kg, 2500 µl/kg, 3020 µl/kg and 3720 µl/kg) to reflect differences seen in the ALD. The dose for each animal was calculated based on the animal's weight, the dose level desired and the concentration of the dosing solution.

All animals were fasted overnight before dosing. All animals received a single dose on 25 August 1981. An 18 gauge, 3-inch gastric lavage needle (Popper and Sons, Inc., New Hyde Park, N.Y.) was used to administer the chemical by gastric intubation. The animals were intubated without being sedated or anesthetized.

Observations

Animals were observed daily during the quarantine period. During the course of the study, animals were observed for clinical signs of toxicity at 0730 and 1530 for the first week and at 0730 the remaining week. Findings are reported later in this report.

Duration of Study

The actual study lasted 14 days; however, animals were quarantined and acclimated for 12 days before the study began.

Historical Listing of Study Events

13 Aug 81	40 male and 40 female rats arrived. They were sexed, observed for illness, ear tagged and housed in the GLP Suite.
14 Aug 81	1 male and 1 female rat were submitted to pathology for quality control.
17-21 Aug 81	Rats were predosed daily with 1 cc water.
21 Aug 81	Rats were removed from quarantine status, weighed, observed for illness and randomized into groups.
24 Aug 81	Feed was removed at 1800 hr.
25 Aug 81	Rats were weighed and dosed according to group.
25-29 Aug 81	Clinical signs recorded at 0730 and 1530 hr daily.
28 Aug, 4 Sep 81	Animals were weighed.
30 Aug - 9 Sep 81	Clinical signs were recorded at 0730 hr.
7 Sep 81	Feed removed from males.
8 Sep 81	Feed removed from females. Males rats that survived were sacrificed by euthanasia and gross pathological necropsies performed.
9 Sep 81	Female rats that survived were sacrificed by euthanasia and gross pathological necropsies were performed.

Changes to Original Objectives and Procedures

1. Analysis of the chemical (CHR 8) was not performed. Chemical analyses were waived due to the cost of development and the confidentiality of the compound.

2. During dosing, animals D8100315 (male-group 4), D8100377 (female-group 6), and D8100378 (female-group 2) did not receive their full dose; consequently, they were not included in summarizing the results. Animal D8100343 was eliminated from LD_{50} determination after necropsy due to a fractured femur of unknown traumatic origin.

3. On 22 August 1981 the humidity in the animal room was peaked for approximately 1 hour and was elevated for approximately 6 hours above the range specified under Environmental Conditions. This was due to a routine steam outage supervised by the building engineers and did not affect the course of the study.

4. During randomization using the Animal Allocation System, the weights of four male rats (D8100308, Group 6, D8100307, Group 2, D8100334, Group 4 and D8100331, Group 3) were more than 20% variant from the overall mean. There were not enough extra animals to eliminate these low weights and the animals were judged to be healthy at study initiation. No statistics are required for loss or gain in weight during these studies. These animals were not eliminated from the study.

RESULTS

Mortality

Table 1 lists the compound related deaths by group for male rats.

TABLE 1
Compound Related Deaths by Group
Male Rats

Group	Dose Level (l/kg)	Compound Related Death/ Number in Group
1	Vehicle Control	0/5
2	2290	0/7
3	2820	0/7
4	3500	1/6[a]
5	4270	0/7
6	5250	5/6[b]

a - misdose; b - fractured femur

Table 2 lists the compound related deaths by group for female rats.

TABLE 2
Compound Related Deaths by Group
Female Rats

Group	Dose Level (µl/kg)	Compound Related Death/ Number in Group
1	Vehicle Control	0/5
2	1620	0/6[a]
3	2000	0/7
4	2500	1/7
5	3020	4/7
6	3720	4/6[b]

a - misdose, survived 14 day period.
b - misdose, collapse 24 hours, dead 28 hours after dosing.

Slope Determination

A Fortran V Program on a Data General Eclipse C/330 Computer was used to perform Bliss' method of probit analysis for the number of animals dead per group (1). The program utilized the percentage kills to determine the weighted regression line of the mortality probit on the log-dose, which results in the formulas:

$$\text{Males } Y = -39.855 + 12.18\ X$$

$$\text{Females } Y = -24.41 + 8.4\ X$$

where Y is the probit and X is the logarithm of the dose. A Chi square statistic was calculated to test the acceptability of the line at the 0.05 significance level (x^2 male = 5.1; x^2 female = .75). The probits were then converted back to percentages and the LD_1, LD_{50} and LD_{95} determined along with their 95% confidence limits. The probit analysis checked the Chi square and found it acceptable for the male and female lines. The slope of the female line, because of the irregularity of the dose group size (i.e., 7 in group 5, and 6 in group 6) determined that the "assay may be meaningless". This is not the investigator's opinion, however.

Lethal Dose Calculations

Lethal dose (LD) values calculated by probit analysis for CHR 8 are given below for males (Table 3) and females (Table 4).

TABLE 3
Lethal Dose (LD) Levels of CHR 8 in Male Rats

Percent Population	Lethal Dose (µl/kg)	95% Confidence Interval (µl/kg)
LD_1	3097	813.9 - 11,797
LD_{50}	4810	4035 - 5733
LD_{95}	6564	1727 - 24,944

TABLE 4
Lethal Dose (LD) Levels of CHR 8 in Female Rats

Percent Population	Lethal Dose (µl/kg)	95% Confidence Interval (µl/kg)
LD_1	1659	521 - 5286
LD_{50}	3136	2654 - 3706
LD_{95}	4920	1948 - 12,430

Figure 1 is a graphic representation of the probit analysis derived response curves for males and females. In Figures 2 and 3 the response curves for males and females were graphed separately with the respective 95% confidence intervals.

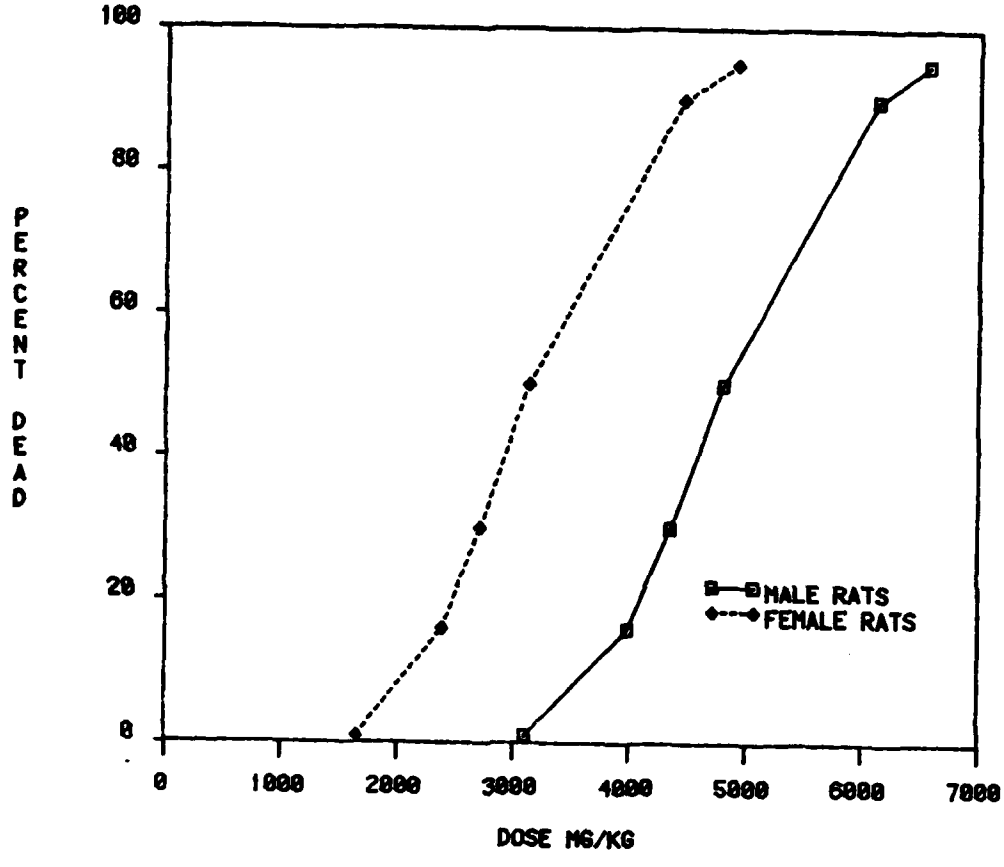

Figure 1: Probit analysis derived dose response curve for males and females in GLP Study 81022

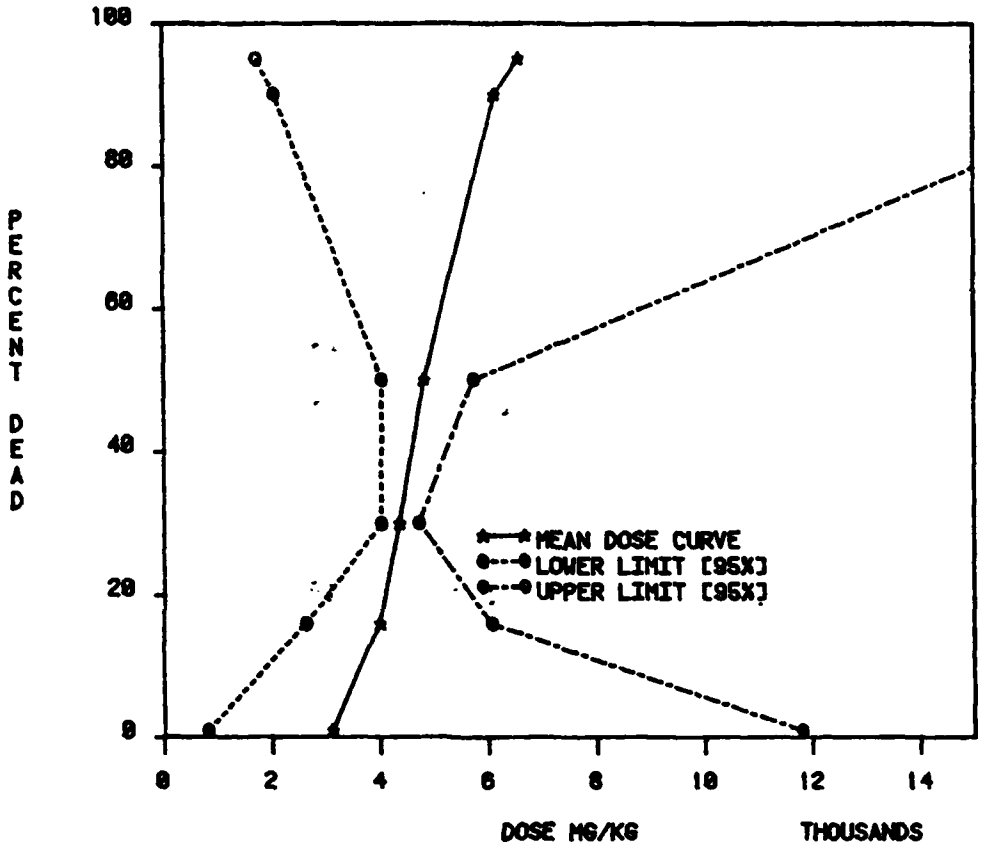

Figure 2: Probit analysis derived dose response curve for CHR 8 males in GLP Study 81022

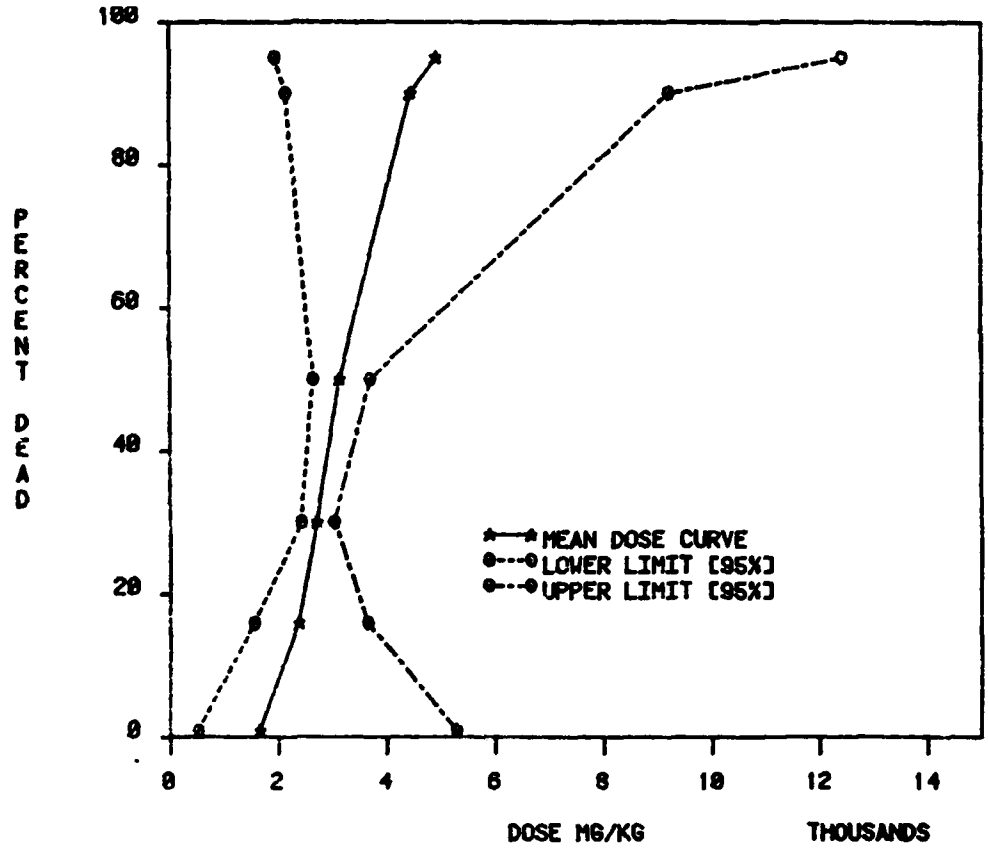

Figure 3: Probit analysis derived dose response curve for CHR 8 females in GLP Study 81022

Clinical Observations

For the first week, animals were observed twice a day in undisturbed cages, outside of cages and after replacement in cages. Recorded observations were approximately 12 hours apart. A quantitive summary of the incidence of clinical signs was prepared, although statistical analysis of the signs was not performed.

Males: Clinical signs were summarized for male rats exposed to CHR 8. Group 5 (4270 µl/kg) experienced no losses out of seven animals assigned to that group. However, in Group 4 (3500 µl/kg) one animal died and in Group 6 (5250 µl/kg) 5 of 6 animals died due to chemical intoxication. Clinical signs for Group 6 were scant and/or represented agonal changes. Changes in respiratory rate (decrease) in depth (increase) and pull reflex depression were more marked in Groups 4 and 6 than in Groups 3 (2820 µl/kg) or 5. Piloerection and a humpback attitude was seen in at least two animals in each of the top four dose groups. Inactivity and sluggishness plagued all dose groups and affected more than 50% of the top three dose groups. A few animals in the vehicle control group and the lower dose groups (excluding the highest dose group) were noted as irritable.

Harderian and other glandular secretions were most often recorded as "red material" or "red stain" and were noticed in all dose groups anywhere from 3 of 7 to 6 of 6 animals, especially on the forepaws and faces of the animals. Yellow and orange stains, assumed to be urinary or fecal excrement (with or without metabolic by products of the test compound) were seen in all dose groups.

This represents, perhaps, a lethargy on the part of the exposed animals to undergo normal grooming behavior, and possibly the ability of the compound or its by products to stain rat hair.

Four animals in Group 6 were observed in a collapsed state before death was recorded. One male was described as collapsed 24 hours after dosing, and then slightly sluggish for up to 32 hours, then he collapsed again with severe chronic convulsions and was found dead at the next observation period (Group 6). One animal in Group 4 collapsed and recovered.

Females: Death was seen in the top three dose levels. Dose groups were not the same as for males, (see Dosing). Two animals collapsed and recovered (1 in Group 4, 1 in Group 6). Respiratory changes were slight, and were seen mostly in Group 5. Two animals demonstrated convulsions. One animal collapsed and died about 35 hours after convulsions were noticed (Group 5). One female was seen convulsing after collapse but recovered. Female animals experienced more serous (clear fluid) ocular tearing than males. Eyes remained open in the comatose state (personal observation). Piloerection, reflex changes, equilibrium changes and staggering was seen in

moderate to low frequency in most dosed groups. The syndrome of inactivity and sluggishness was prevalent in Groups 3 through 6. Red material around the head and forepaws was frequently seen as was the orange/yellow perineal staining - especially in Group 4 animals. The explanations for this occurrence is the same as for males. All animals that died from the chemical, died within three days after dosing. The greatest incidence of deaths among female rats was 40 $\pm$ 5 hours after dosing. Male rats lingered longer with the highest number of deaths occurring approximately 70 $\pm$ 5 hours after dosing. The exact cause of death is not known, but gross pathological examination revealed gross lesions in the gastrointestinal tract and lungs. Figure 4 demonstrates the time of death for males and females and the respective dose groups.

Gross Pathological Observations

The Pathologist's Report appears in Appendix B.

Necropsy Procedures: Animals found dead in the morning were placed in the refrigerator and a necropsy was performed by the pathologist on call. Animals were not dead for more than twelve (12) hours before necropsy. Necropsy was performed in accordance with SOP OP-STX-32.

DISCUSSION

Clinical observation data sometimes appears slanted for several reasons: 1) Animals in the high dose groups often die, giving only moribund or agonal signs. 2) A sign that is considered marked one day and slight the next is considered to be two separate kinds of observations. Slight changes in respiratory patterns, for instance, are less meaningful than marked changes. These changes may occur in the same animal and thus this summary of a clinical sign that include slight, moderate and marked may indicate that more than 100% of the group demonstrated that sign. 3) Observations of clinical signs are subjective measurements of toxicity and are only conducted at 12-hour intervals. The purpose of recording the clinical signs was to gain more information on the toxic effects of the formulation for use in future studies. The clinical course of the intoxication can be studied more reliably in subchronic and chronic studies.

The calculated LD_{50} for CHR 8 in male rats was 4810 µl/kg with a 95% confidence interval of 4035-5733 µl/kg. The LD_{50} for CHR 8 in female rats was 3136 µl/kg with a 95% confidence interval of 2654 to 3706 µl/kg. The evaluation of the toxicity of a chemical after the establishment of the LD_{50} in rats is based on objective and subjective interpretations. According to the FDA, man is ten times more sensitive to a drug at a given drug dose ratio to weight ratio as a

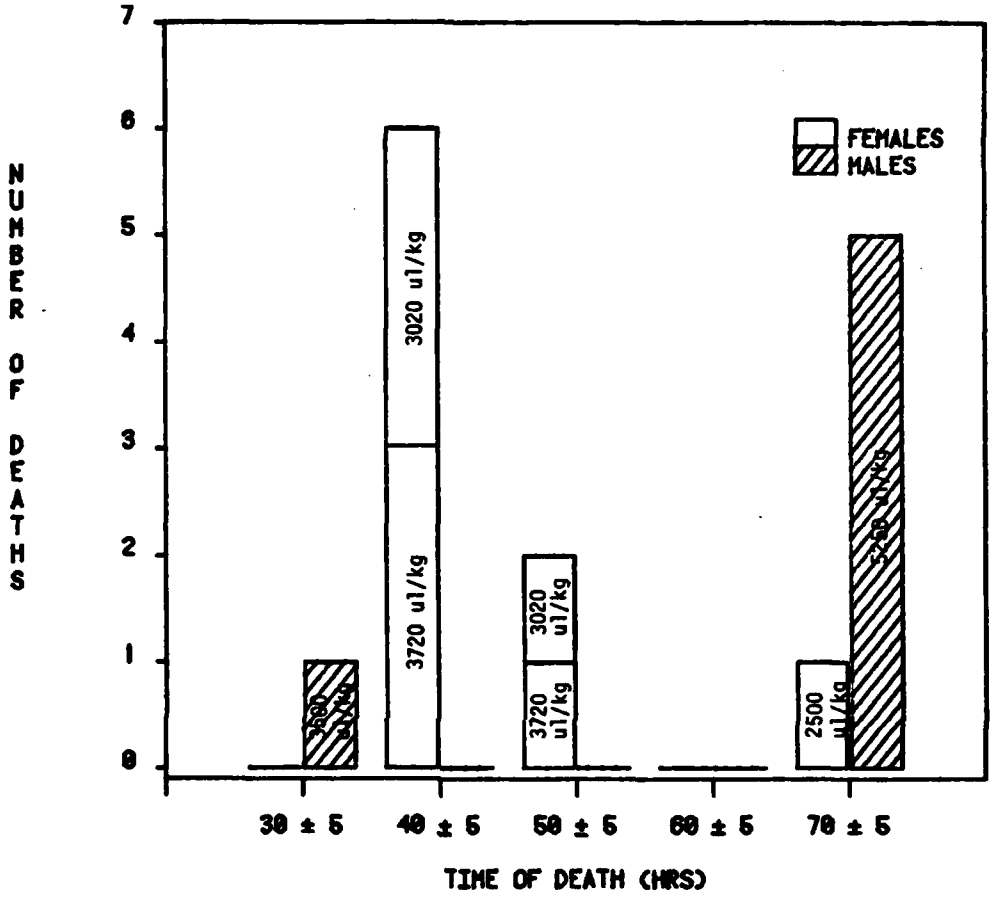

Figure 4: Time of death for male and female rats exposed to CHR 8 in GLP Study 81022

rat. This assumes a 60 kg man and a 0.4 kg rat with a weight ratio to man of 1:150 and a drug dose ratio of rat/man of 1:15 (2).

Two sources classify chemicals according to their toxicity. Classification appears in Table 5.

TABLE 5

Classification of Toxicity

LD_{50} level (mg/kg)	Nat'l Research Council (3)	Zbinden et al (4) and Gleason et al (5)
< 1	extremely toxic	
< 5		super toxic
5-50		extremely toxic
1-50	highly toxic	
50-500	moderately toxic	very toxic
500-5,000	slightly toxic	moderately toxic
5,000-15,000	practically non-toxic	slightly toxic
> 15,000	harmless	practically non-toxic

From Table 5, it is obvious that a chemical with an LD_{50} of 5 g/kg could be considered at a liberal estimate practically non-toxic (3) or at a conservative estimate moderately toxic (4,5), a magnitude of up to and including 30 to 300 times difference. The slope of the line produced from the probit analysis demonstrates the ratio between the change in dose and the change in response or mortality. A flat dose-mortality curve suggests a potential for cumulative toxicity, and a potential of occasional instances of extreme susceptibility to the chemical. A 'steep' curve indicates little variability and permits a better estimate of safe dosage.

The LD_{50} and slope of the line for CHR 8 in rats suggests to this laboratory a slightly toxic chemical and one with little intraspecies variabliity from this test. Further testing in other species would serve to facilitate the extrapolation of these mortality results for man.

CONCLUSION

The LD_{50} for CHR 8 is 4736 µl/kg for male and 3136 µl/kg for female rats.

RECOMMENDATION

Toxicity testing of CHR 8 should continue for eventual human use screening.

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3. COMMITTEE FOR THE REVISION OF NAS. Publication 1138. COMMITTEE ON TOXICOLOGY, NATIONAL RESEARCH COUNCIL. Principles and Procedures for Evaluating the Toxicity of Household Substances. Washington DC: National Academy of Sciences, 1977

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5. GLEASON, M.N., GOPELIN R.E., HODGE H.C., SMITH R.P. Clinical Toxicology of Commercial Products, Acute Poisoning (Third edition) Baltimore: Williams & Wilkins Co., 1973

APPENDICES A and B

1. Chemical Name: Confidential

Chemical Abstract Service Registry No.: Unknown

Molecular structure: Unknown

Molecular weight: Unknown

pH: N/A non-aqueous

Physical state: liquid

Boiling point: Unknown

Compound density: Unknown

Compound refractory index: $n_D^{20} = 1.5212$

Stabiliity: Unknown

Contaminants: Unknown

Manufacturer: Rohm & Haas, Cutter Laboratories
4th and Parker
Berkeley, California 94719

Manufacturer Lot No: Unknown

APPENDIX A

Gross Pathology Summary and Interpretation of GLP Study 81-022, LD_{50} CHR8, A Candidate Insect Repellent, Male Sprague-Dawley Rats

The deaths of 5/6* male rats in group 6 (5250 ul/kg)** and 1/6 rats in group 4 (3500 ul/kg) were attributed to the toxic effect of the tested compound. All of the compound related deaths were observed between 28 hours and 20 minutes and 69 hours and 4 minutes after gastric intubation with test compound. None of the male rats in group 1 (controls), group 2 (2290 ul/kg), group 3 (2820 ul/kg), or group 5 (4270 ul/kg) died prior to termination of the study.

Gross changes attributable to the test compound were present in the digestive system of the rat in group 4 and 1 of the rats in group 6 that died. Both rats had small intestines that were distended with yellow/white mucoid material, and the rat in group 6 had several red-brown foci in the glandular portion of its stomach that probably represented erosions or petechial hemorrhages. Some of the changes may have been exaggerated by autolysis.

Red material was encrusted on the hair around the muzzle of all rats that died in group 6. Two of these rats had similar material on their forepaws and the other 3 had the material around their eyes. This material may have been test compound or one of its metabolites that were being excreted by the lacrimal glands or most likely was due to porphyrins that were being secreted by the Harderian gland in excessive quantities as a response to an irritant. The test compound may have caused the excessive production of porphyrins.

The gross findings of a focal dermal abrasion on the scrotum of 1 rat in group 6, wet perineal area in 2 rats in group 6, a focal cortical cyst in the kidney of 1 rat in group 5, a focal red-brown nodule in the spleen of 1 rat in group 2 and a focal translucent subcapsular nodule in the spleen of 1 rat in group 1 are considered to be incidental lesions that were not related to the administration of the test compound.

The additional rat in group 6 that was observed dead 27 hours 59 minutes after gastric intubation had a fractured femur that was surrounded by a large blood clot. The distention of the small intestine and stomach with yellow/white mucoid material, the red

*Number of rats affected/Number of rats in the group

**An additional rat in group 6 that died had a fractured femur that was surrounded by a large blood clot. This rat was removed from consideration for the purpose of data evaluation in this gross pathology report.

APPENDIX B

mottling of the lungs, and the presence of red material on hair around the eyes and muzzle may have been due to the test compound. The clouded corneas in this rat are considered to be incidental findings that are not attributed to the test compound. It is impossible to predict if this rat would have lived or died if its leg had not been fractured.

In summary, the gross pathologic effects, in addition to death, that most likely were due to single dose gastric intubation with CHR8 that were observed in the male Sprague-Dawley rats used in this study are:

1. Erosions or petechial hemorrhage in the stomach.

2. Excess glandular secretion in the stomach and small intestine.

3. Excess secretion by lacrimal glands, most likely the Harderian glands.

Necropsies revealed no test compound related lesions in the male Sprague-Dawley rats that were killed at the termination of the study.

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APPENDIX B

Gross Pathology Summary and Interpretation of GLP Study 81-022, LD_{50} CHR8, A Candidate Insect Repellent, Female Sprague-Dawley Rats

The deaths of 4/6* female rats in group 6 (3720 ul/kg), 4/7 rats in group 5 (3020 ul/kg), and 1/7 rats in group 4 (2500 ul/kg) were attributed to the toxic effect of the tested compound. All of the deaths were observed between 44 hours 20 minutes and 68 hours 32 minutes after gastric intubation with test compound. None of the female rats in group 1 (controls), group 2 (1620 ul/kg), or group 3 (2000 ul/kg) died prior to termination of the study.

Gross changes attributable to the test compound were present in the digestive system of 2 rats in group 5 and 3 rats in group 6 that died. The small intestines of the 3 rats in group 6 were filled with yellow-mucoid material and the small intestines of 1 rat in group 5 were filled with yellow/black material that probably contained blood from the stomach. The stomach of 2 rats in group 5 had several red/brown foci in the glandular portion. These foci probably represented erosions or petechial hemorrhages. Some of the changes may have been exaggerated by autolysis.

Red material was encrusted on the hair around the muzzle of 3 rats in group 6, 2 rats in group 5 and the 1 rat in group 4 that died. All of the rats with red material on the muzzle and an additional rat in group 5 had similar material around their eyes. This material may have been test compound or one of its metabolites that were being excreted by the lacrimal glands or most likely was due to porphyrins that were being secreted by the Harderian glands in excessive quantities as a response to an irritant. The test compound may have caused the excessive production of porphyrins.

The yellow stained perineal hair on 3 rats in group 6, 4 rats in group 5, and 1 rat in group 4 that died may have been due to the urinary excretion of the test compound or its metabolites.

The gross findings of multifocal erosions on the tail of 1 rat in group 5 and 1 rat in group 4, corneal opacities in 2 rats in group 5, a unilateral dilated renal pelvis in 1 rat in group 4, enlarged bronchial lymph nodes in 1 rat in group 3, and a focal skin abrasion near the vulva of 1 rat in group 2 are considered to be incidental lesions that were not related to administration of the test compound.

Generalized autolysis was present in 3 of the rats in group 6 and 3 of the rats in group 5 that died.

*Number of rats affected/Number of rats in the group

APPENDIX B

In summary, the gross pathologic effects, in addition to death, that most likely were due to single dose gastric intubation with CHR8 that were observed in the female Sprague-Dawley rats used in this study are:

1. Petechial hemorrhage and/or erosions in the stomach.

2. Excess glandular secretion in the stomach and small intestine.

3. Excess secretion by lacrimal glands, most likely the Harderian glands.

4. Yellow staining of perineal hair.

Necropsies revealed no test compound related lesions in the female Sprague-Dawley rats that were killed at the termination of the study.

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